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Identification of a non-native *Cynoscion* species (Perciformes: Sciaenidae) from the Gulf of Cádiz (southwestern Spain) and data on its current status

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Summary: *Cynoscion* is a genus of fish in the family Sciaenidae from the Atlantic and Pacific American coasts that is important in recreational and commercial fisheries. Morphological analysis identifies a species inhabiting the Gulf of Cádiz (southern Spain) as the weakfish, *Cynoscion regalis*, a native of the northwest Atlantic. This finding is also confirmed by molecular identification using 16S and Cox1genes. Based on the examination of a previous manuscript, the assignation of this species to the spotted seatrout, *Cynoscion nebulosus*, is considered a misidentification. *C. regalis* has been reported in the area since 2011 and is now considered an established species that is distributed along the Guadalquivir River estuary and is a target of local artisanal fisheries. The pathway of introduction is unknown, but possible mechanisms are considered, of which ballast water seems to be the most plausible. A revision of non-native sciaenids also found in European waters is carried out. The ecological impact of weakfish on the local fish community is still unknown and should be object of future studies.

Keywords: biological invasion; marine introduction; weakfish; *Cynoscion regalis*; Guadalquivir estuary; taxonomy.

Identificación de una especie no nativa de *Cynoscion* (Perciformes: Sciaenidae) en el Golfo de Cádiz (suroeste de España) y datos sobre su estado actual

Resumen: *Cynoscion* es un género de peces de la familia Sciaenidae, de importancia en la pesca deportiva y comercial, que habita las costas americanas del Atlántico y el Pacífico. Una especie que habita en el Golfo de Cádiz (suroeste de España) fue identificada mediante análisis morfológico como corvinata real *Cynoscion regalis*, nativa del Atlántico Noroeste. La identificación también fue confirmada por análisis molecular, utilizando los genes 16S y Cox1. Basándose en el examen de una publicación anterior, la asignación de esta especie a corvinata pintada *Cynoscion nebulosus* se considera un error de identificación. Existen registros de *C. regalis* en esta zona desde 2011 y ahora se considera una especie establecida, distribuida a lo largo del estuario del río Guadalquivir y objeto de la pesca artesanal local. La vía de introducción es desconocida, pero se consideran los posibles mecanismos de introducción, de los cuales el agua de lastre parece ser el más probable. Se realizó una revisión de los esciaénidos no nativos en aguas europeas. Aún se desconoce el impacto ecológico de la corvinata real en la comunidad local de peces, que debe ser objeto de futuros estudios.

Palabras clave: invasión biológica; introducción de especies marinas; corvinata real; *Cynoscion regalis*; estuario del Guadalquivir; taxonomía.

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INTRODUCTION

The family Sciaenidae comprises approximately 70 genera and 270 species of demersal fishes that are commonly known as drums and croakers, found mainly over muddy or sandy bottoms of tropical, subtropical and warm temperate regions of the Atlantic, Indian and Pacific oceans, from the shoreline up to about 600 m depth (McEachran and Fechhelm 2005, Nelson 2006). Most species are marine, a few are euryhaline and support a wide range of salinities, and 25 species, mostly from South America, are strictly freshwater (Casatti 2002).

The eastern Atlantic, with 19 sciaenid species, is the least diverse geographical area, compared to the eastern Pacific, with 82 species, the western Atlantic, with 82, and the Indo-West Pacific, with 92 (Lo et al. 2015). Only five native species occur in Iberian and Atlantic European waters: *Argyrosomus regius* (Asso, 1801), *Sciaena umbra* Linnaeus, 1758, *Umbrina canariensis* Valenciennes, 1843, *Umbrina cirrosa* (Linnaeus, 1758) and *Umbrina ronchus* Valenciennes, 1843 (Quéro et al. 2003, Lloris 2015).

Two introduced sciaenids species have also been reported in Atlantic European waters. Two living specimens of the Atlantic croaker, *Micropogonias undulatus* (Linnaeus, 1766), were reported in Belgian waters in 1998 and 2001 (Stevens et al. 2004) and one fresh specimen of about 28 cm total length, tentatively identified as *M. undulatus*, which was found displayed for sale at Plymouth Fish Market in September 2011 (http://www.glaucus.org.uk/), was probably captured in the southwestern approaches (SW Cornwall). One specimen of the spotted seatrout, *Cynoscion nebulosus* (Cuvier, 1830), was caught in 2011 by a fishing vessel on a sandy bottom near the Guadalquivir River estuary, southern Spain (Acosta et al. 2013).

The genus *Cynoscion* Gill, 1861 is a large, ecologically and economically important genus of marine fishes in the family Sciaenidae that is found throughout tropical and subtropical coastal waters of the New World (Vergara-Chen 2009). Currently, there are 24 recognized species, 12 from the eastern Pacific and 12 from the western Atlantic, inhabiting estuarine and inshore waters, mainly in the tropical and subtropical regions of Central and South America (Chao 2002a).

The Gulf of Cádiz is the sub-basin of the North Atlantic nearest to the Strait of Gibraltar. It is bordered to the north by the southwest coasts of the Iberian Peninsula, to the south by the Atlantic coast of Morocco, to the east by the Strait of Gibraltar, and to the west by the 9°W meridian (Criado-Aldeanueva et al. 2006). There is an important artisanal fishery and shellfishing sector in the area, with a fleet composed of about 1000 vessels that capture more than 50 commercial species (Silva et al. 2002), although current official data reduce the number of vessels by nearly half. Human impacts on this marine ecosystem through exploitation of marine resources, fishing and the invasion of non-indigenous species have also been reported (Chicharo et al. 2009).

There are three general mechanisms through which non-native species may enter a new region: importation as or with a commodity, arrival through a transport vector and dispersal by the species themselves, either along infrastructure corridors (e.g. roads, canals) or unaided (Hulme et al. 2008). For fishes, the most widely reported pathways of introduction are ballast water transport, marine culture, aquarium trade, oil drilling platform transfer, fisheries development, species released for scientific research, and movement through canals (Liao et al. 2010).

Molecular techniques have been successfully integrated with traditional morphological analysis in the identification of fishes, reinforcing the resulting taxonomic identification and reducing the possibility of identification mistakes (Bañón et al. 2013). In addition, the use of DNA barcodes efficiently helps to detect and understand marine invasions through the identification of exotic species and their geographic origin (Bariche et al. 2015).

The present work discusses the current taxonomic status of the *Cynoscion* species found in the Gulf of Cádiz based on morphological and molecular features within an integrative context. The distribution, abundance, possible mechanisms of introduction and ecological consequences of this introduced species are also discussed.

MATERIALS AND METHODS

Sampling, morphological identification and study area

Thirteen specimens were caught with gillnets by local fishermen in the Gulf of Cádiz (SW Spain). The specimens were initially preserved frozen at –20°C. After thawing them, we took measurements and meristic characters to the nearest mm and then preserved them in ethanol (80%) in the laboratory. The identification was made using descriptions and keys reported by Chao (2002a, b). The preserved specimens were deposited at the Colección de Fauna Marina del Centro Oceanográfico de Málaga, with the reference numbers CFM-IEOMA-6020 to 6032.

Molecular identification

DNA barcode sequences were obtained for 3 out of the 13 specimens of *Cynoscion* collected in this study (CFM-IEOMA 6020-6022). The molecular identification was based on partial sequences of the mitochondrial genes 16S RNA and Cytochrome c oxidase subunit I (Cox1). Total genomic DNA was extracted from fin tissue following a modified Chelex 10% protocol by Estoup et al. (1996).

Target mitochondrial DNA from the 16S rRNA and Cox1 genes was amplified with polymerase chain reaction (PCR) using the following cycling conditions: 2 min at 95°C, 40 cycles of 20 s at 95°C, 20 s at 45-48°C, 45 s (16S) or 47 s (Cox1) at 72°C, and 5 min at 72°C. Primers 1472 (5′- AGA TAG AAA CCA ACC TGG -3′) (Crandall and Fitzpatrick 1996) and 16L2 (5′-TGC CTG TTT ATC AAA AAC AT-3′) (Schubart et al. 2002) were used to amplify 611 bp of 16S, while primers COH6 (5′- TAD ACT TCD GGR TGD CCA AAR AAY CA -3′) and COL6b (5′- ACA AAT CAT

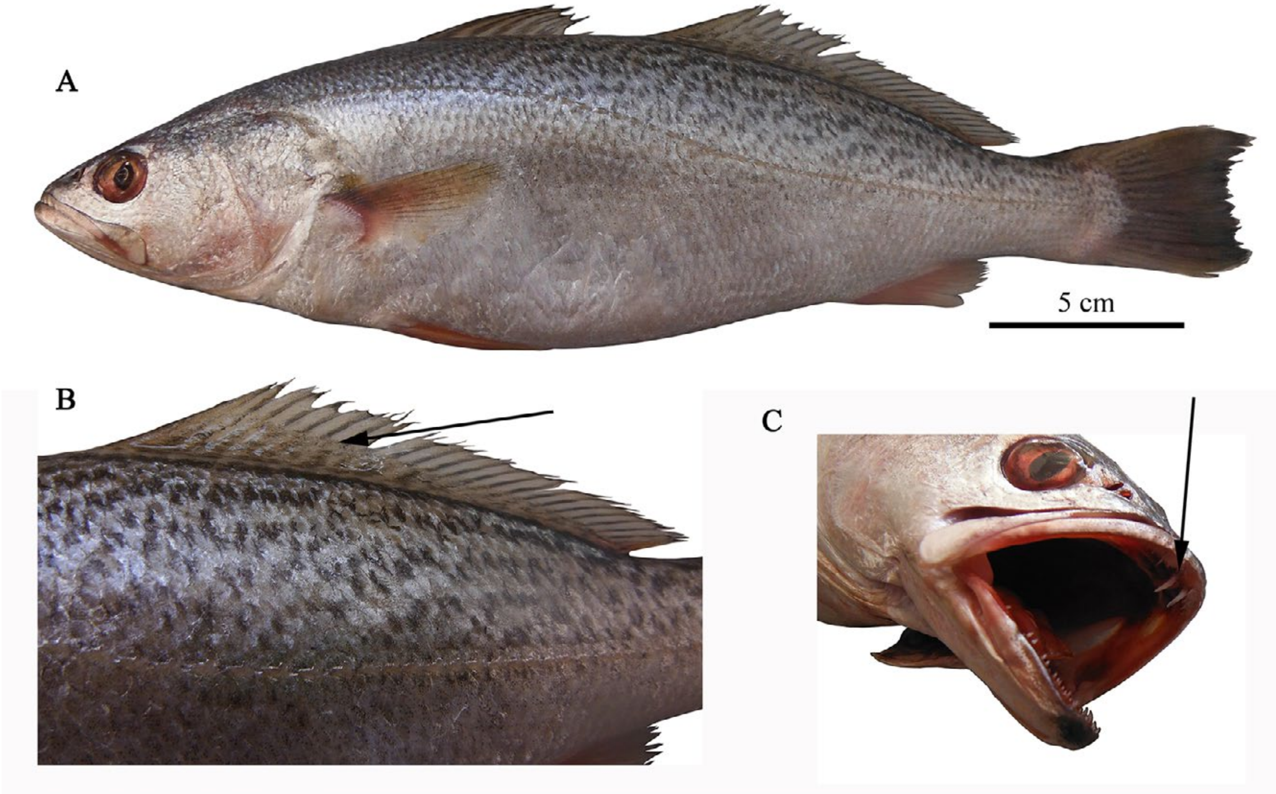

Fig. 1. – Specimen of *C. regalis*. A, view of an entire specimen of 313 mm TL; B, detail of scales at the base of soft dorsal fin; C, detail of the two large canine-like teeth in the upper jaw. The arrows indicate the position of the described character.

AAA GAT ATY GG -3′) (Schubart and Huber 2006) were used to amplify 660 bp of Cox1. PCR products were sent to Stab-Vida Laboratories to be purified and then bidirectionally sequenced. Sequences were edited using the Chromas software, version 2.0. The final DNA sequences obtained were used in a BLAST search executed on the NCBI webpage to get the sequence that matched best.

An evolutionary distances analysis was carried out in MEGA6 (Tamura et al. 2013). Alignment of 16S and Cox1 sequences was carried out using sequences obtained from the specimens studied as well as other congeneric species downloaded from GenBank (http://www.ncbi.nlm.nih.gov). The phylogeny reconstruction analyses were inferred from neighbour-joining using the p-distance method. The nodal confidence of obtained topologies was assessed via 2000 bootstrap replicates.

Fishery data

Catch data were compiled from fishing statistics provided by the Andalusian fisheries authorities and calculated using the sales notes submitted by fish market operators where weakfish was landed and sold. Distribution of *C. regalis* in the Gulf of Cádiz was estimated on landings as well as on-field work carried out at the ports to confirm the presence of weakfish among catches. Skippers from vessels landing weakfish were interviewed in order to obtain more accurate information on where the weakfish was being caught.

RESULTS

Material examined

Cynoscion regalis (Bloch and Schneider, 1801) (Fig. 1), 13 specimens between 276 and 330 mm total length (TL), 234 and 285 mm standard length (SL), 22 February 2016, vessel “Dolores y Juan”, 36°49.736’ N 6°32.922’ W, 17-18 m depth.

Description

Body elongated and moderately compressed, body depth 3.5-4.1 times the SL; predorsal profile nearly straight and ventral concave; head moderate and conical, 3.4-3.7 times the SL; snout pointed with lower jaw projecting; maxillary reaching beyond pupil; upper jaw with a pair of large canine-like teeth at its tip; interorbital width greater than horizontal eye diameter; one continuous and long dorsal fin with a deep notch between spinous and soft-rayed portion; anal fin shorter and placed at the back of the body; scales at the base of soft dorsal and anal fins; gas bladder with a pair of nearly straight, horn-like appendages. Colouration: body silvery, darker above and lighter below; back with small spots forming undulating dotted lines. Pelvic and anal fins are yellowish, while the other fins are pale. The main morphometric and meristic characters are presented in Table 1.

Table 1. – Comparison of morphometric, meristic data and respective body proportions for specimens of *C. regalis*.

Cynoscion regalis	CFM-IEOMA-6020 to 6032 (N=13)	McEachran and Fechhelm 2005
Total Length (mm)	276-330	–
Standard length (mm)	234-285	–
As % SL		
Head length	27.1-28	29-32
Eye diameter	5-6	6-7
Preorbital length	5.9-7.4	7-8
Postorbital length	15.8-16.5	–
Interorbital length	6.1-7.3	5-6
Maxillary length	10.7-12.2	13-14
Predorsal length	30.8-34.5	–
Dorsal base length	50.2-56.0	–
Preanal length	68.4-73.3	–
Anal base length	9.7-11.8	–
Pectoral length	15.4-16.9	13-15
Pelvic length	14.3-16.5	–
Prepelvic length	27.1-29.5	–
Prepectoral length	24.2-27.6	–
Body depth	24.2-28.3	24-25
Meristic features		
Dorsal fin rays	X+I+26-29	X+I+24-29
Anal fin rays	II+11-12	II+10-13
Ventral fin rays	I+5	–
Pectoral fin rays	18-20	18-19
Pored scales lateral line	54-59	–
Branchiostegal rays	7	–
Gill rakers	5-6+1+10-11	14-20

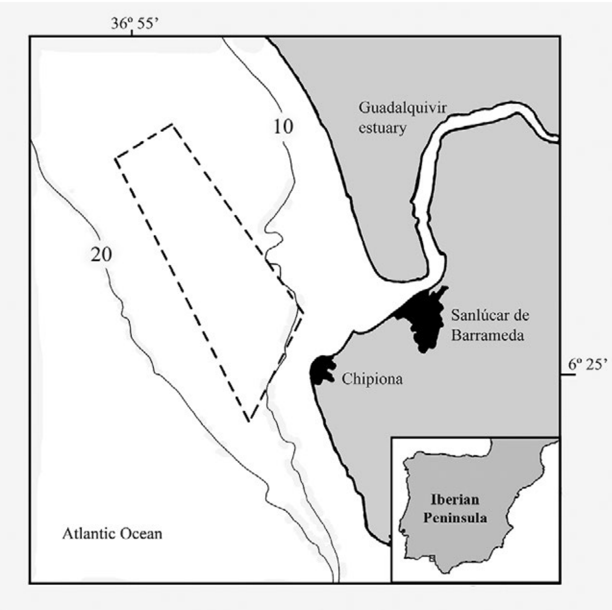

Fig. 2. – Map showing the main distribution area, framed by dashed line, of *C. regalis* in the Gulf of Cádiz based on fisheries data.

Native distribution

They are found on the Atlantic coast of North America from Nova Scotia to south Florida and on the western coast of Florida (uncommon) (McEachran and Fechhelm 2005), but they are most abundant off the coasts from North Carolina to New York (Lowerre-Barbieri 1996).

Exotic distribution

A juvenile specimen of *C. regalis* of 98 mm TL was captured on 24 September 2009 by fyke net in the Schelde Estuary (Belgium) and the identification was confirmed by DNA analysis and published in Dutch in a local sport fishing magazine (Agentschap voor Natuuren Bos 2011). Recently this species was also caught in Portugal, in the Sado estuary (Béarez et al. 2016). In the Gulf of Cádiz, this species has been found between the cities of Cádiz and Ayamonte, although most catches come from the Guadalquivir estuary, mainly over muddy bottoms, close to the fishing villages of Chipiona and Sanlúcar de Barrameda (Fig. 2).

Molecular identification

The three 16S sequences of 611 bp obtained (GenBank accession numbers KX879594, KX879595 and KX879596) have the same haplotype as well as the three Cox1 sequences of 660 bp (GenBank accession numbers KX879597, KX879598 and KX879599).

In the obtained trees, the sequences of studied specimens cluster in strong supported clades with one sequence of 16S of *C. regalis* (Fig. 3) and eight sequences of Cox1 of *C. regalis* (Fig. 4), and in both cases are clearly separated from the closely related congeneric species *Cynoscion arenarius* Ginsburg, 1930.

Fishery and abundance

This species has been caught sporadically since at least 2011, with regular catches since November 2015 and declared catches since February 2016 (Table 2) due to initial misidentification as *A. regius*. As misidentification continues among fishermen and fish market operators due to morphological similarities with *A. regius*, catches are likely to be greater than the declared values shown in Table 2. Weakfish is not a target species, being mainly caught by gillnets of 60-70 mm mesh size directed to *Merluccius merluccius* (Linnaeus, 1758) and *Pagellus* sp. and secondarily by bottom trawl, mainly over muddy bottoms. Declared

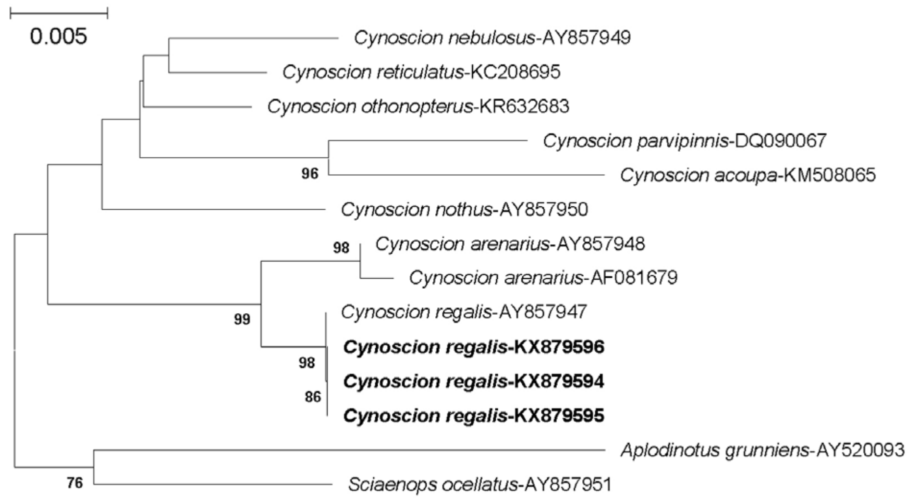

Fig. 3. – Topology of Neighbour-joining tree based on 611 bp of the 16S gene sequences, showing inferred phylogenetic relationships within the genus *Cynoscion* with *Aplodinotus grunniens* and *Sciaenops ocellatus* as outgroups. Numbers close to nodes indicate bootstrap support (only values above 50% shown). GenBank accession numbers are shown after name of species.

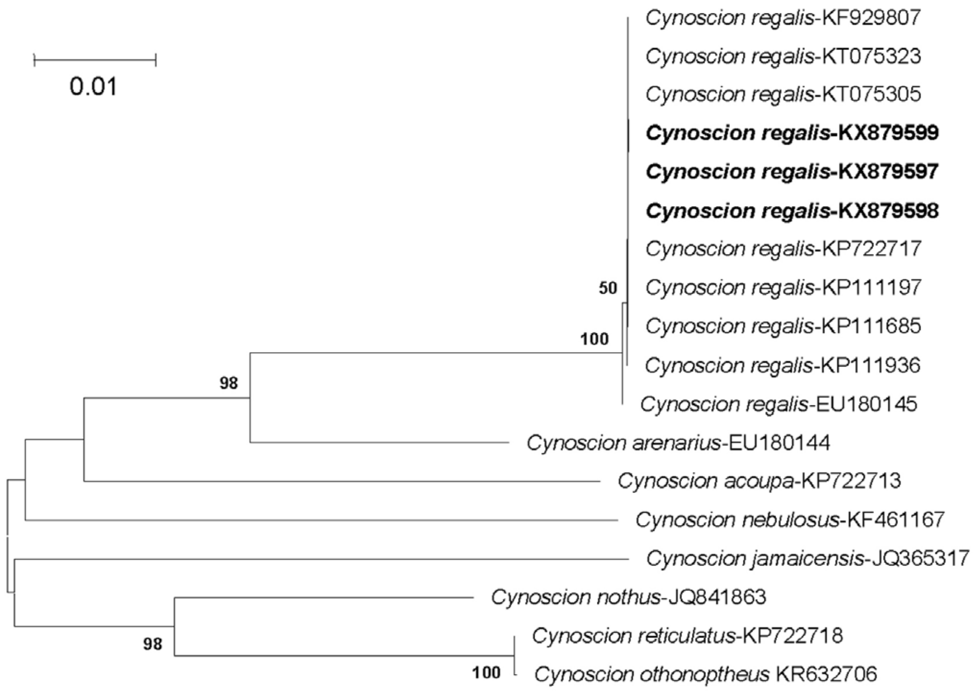

Fig. 4. – Topology of neighbour-joining tree based on 660 bp of the Cox1 gene sequences, showing inferred phylogenetic relationships within the genus *Cynoscion*. Numbers close to nodes indicate bootstrap support (only values above 50% shown). GenBank accession numbers are shown after name of species.

Table 2. – Declared landings (in kg) of *C. regalis* per month in ports of the Gulf of Cádiz in 2016.

Month	Chipiona	Sanlúcar	Rota	Puerto Sta. María	Weight
February	489.05	66.85	8.90	—	564.80
March	516.25	120.40	31.90	11.88	680.43
April	17.95	—	1.70	10.56	30.21
Total	1023	187	43	22	1275

catches by month ranged from 30 kg in April to 680 kg in March. Habitual sizes ranged from about 25 to 35 cm TL, but specimens up to 50 cm TL were also caught.

Fishermen have no concern about this new species since no evidence of negative effects have been reported to date. Weakfish is being marketed locally as *A. regius* although dockside prices are slightly lower than those of *A. regius*.

DISCUSSION

The morphological measurements and meristic counts of the examined specimens identify the species as *C. regalis*, a finding which is also strongly supported by the molecular identification.

The presence of scales at the base of the soft dorsal and anal fins, the colouration (mainly the colour and arrangement of the spots; Fig. 1), biometrics and meristic counts distinguish this species from other sciaenid species. Slight differences found in some biometrics (Table 1) could be due to the lower number of individuals or incomplete size range reported in the literature.

Acosta et al. (2013) reported one specimen of the congeneric *C. nebulosus* in the same area, near the Guadalquivir River estuary (southern Spain). However, a detailed revision of this manuscript showed an incomplete description of the specimen and, finally, an erroneous identification. In their description, the authors omitted an important distinctive character of the species. The presence/absence of scales at the base of soft dorsal and anal fins is a diagnostic character which distinguishes *C. nebulosus* (absence) from the similar *C. regalis* (presence) (Chao 2002a, b, Lassuy 1983). Although Acosta et al. (2013) reported the presence of spots on the caudal and dorsal fins characteristic of *C. nebulosus*, this aspect is not visible in the attached figure. *C. nebulosus* has distinct spots, comparatively fewer and bigger than those in *C. regalis*, and randomly scattered, whereas in *C. regalis* they form oblique and undulating lines (Chao 2002a, b). However, the figure of Acosta et al. (2013) shows a distribution pattern of spots typical of *C. regalis*. Moreover, the reported ten gill rakers count on the lower limb of the gill arch and the pectoral fin slightly longer than the pelvic fin also agree with the description of *C. regalis*, whereas *C. nebulosus* has 6-9 gill rakers on the lower limb of the gill arch and its pectoral fin is shorter than the pelvic fin (Lassuy 1983, Chao 2002a, b).

Molecular identification (16S and Cox1 genes) confirms the previous morphological identification of these specimens as *C. regalis*. The 16S sequences obtained for the three specimens (Fig. 3) present the same haplotype, which fits 100% with the sequence of *C. regalis* deposited in GenBank under the accession number AY857947 and also 100% with a shorter sequence of *C. regalis* (390 bp) deposited in GenBank under the accession number EF095595, obtained in the context of a phylogenetic study of the percoid family Gerreidae (Chen et al. 2007).

In the case of the Cox1 sequences (Fig. 4), the three specimens also have the same haplotype, which fits 100% with four sequences of *C. regalis* deposited in GenBank under the accession numbers KT075323, KT075305, KF929807 and KP722717, belonging to different specimens collected in the US Atlantic waters, and also 100% with three sequences (KP111936, KP111685, KP111197) identified as Actinopterygii environmental samples of eggs attributed through barcoding to *C. regalis* in a study integrating DNA barcoding of fish eggs into ichthyoplankton monitoring programmes (Lewis et al. 2016). In GenBank there is only one more Cox1 sequence, identified as belonging to *C. regalis* (EU180145), which differs in just one mutation from the other seven (GenBank) and three (present study) sequences. This sequence belongs to specimens collected in Delaware (North Carolina) by Seyoum et al. (2013).

After the re-examination of the Acosta et al. (2013) manuscript, we conclude that *C. nebulosus* reported by these authors is a misidentification of *C. regalis*. Thus, this is the only introduced sciaenid species reported until now in the Gulf of Cádiz. The presence of *C. regalis* in the Gulf of Cádiz confirms its presence in Atlantic European waters.

Fishes are one of the largest contributors to the total number of non-indigenous species documented in European seas, accounting for 16.8% of the 879 multicellular non-indigenous species reported by Galil et al. (2014). However, most of these fish species are found in the Mediterranean, introduced via the Strait of Gibraltar (Atlantic species) and the Suez Canal (Lessepsian species) (Oral 2010). In the Atlantic European waters, including the Gulf of Cádiz, the most frequent case is the arrival of southern species by natural displacement due to global warming and changes in the oceanic conditions (Farias et al. 2012, Bañón et al. 2014). In both the Mediterranean (excluding Lessepsian species) and the Atlantic, only a few cases of rare fish species introduced from large distances are documented, such as the South American fish *Pinguipes brasilianus* Cuvier, 1829 in the Mediterranean (Orsi-Relini 2002) and the aforementioned *M. undulatus* in the Atlantic.

As in most cases of marine alien species, the introduction vector cannot be clearly identified. *C. regalis* is highly dependent on estuaries for food, shelter and spawning (Mercer 1989), so it is very unlikely that this species would have swam across the Atlantic Ocean to Spain. However, we cannot rule out this possibility.

Among the anthropogenic pathways of introduction, fishes have been undersampled in ballast water studies and the role of ballast transport in promoting fish invasions has probably been underestimated (Wonham et al. 2000). There are two major ports near the Guadalquivir estuary: the American naval station in Rota, located 20 km from Chipiona, and Sanlúcar de Barrameda, where most of the weakfish is caught. The station has a seaport and provides support to US and NATO ships, and not only military ships. In addition, the port of Cádiz, located some 25 km from Chipiona, features four commercial docks plus a shipyard that repairs ships from all over the world. To support this theory on the introduction of weakfish, some marine organisms are believed to have been introduced through ship ballast water in the Gulf of Cádiz (Cuesta et al. 2004, Chicharo et al. 2009) and the occurrence of the other European alien sciaenid *M. undulatus*, reported in Belgian waters, is likely to be due to transportation in ship ballast water (Stevens et al. 2004) as well.

Aquaculture is the other main vector of introduction of alien species and is the reported pathway of the Atlantic American scianid *Sciaenops ocellatus* (Linnaeus, 1766) on the western Taiwanese coast (Liao et al. 2010). However, as far as we know, the culture of weakfish is only in initial phases at global level, and there is no culture of this species in Spanish waters. Other possibilities of its introduction are very unlikely and were not taken into account. Therefore, as there is no culture data available, introduction by ballast water seems to be the most plausible introduction pathway.

All data suggest that weakfish has survived well and has successfully established populations in the Guadalquivir estuary. The species has been present in the area for at least the last five years. It is very abundant and now forms part of the bycatch of coastal fishers, with declared catches of more than one tonne during three months (Table 2). The population is composed of different year classes and most records are larger than the estimated mean length at first maturity, established at age1 and 164 mm TL for males and 170 mm TL for females (Lowerre-Barbieri et al. 1996), so they would be able to spawn.

Introduction and spreading of alien species are considered one of the main threats to biodiversity. Invasive species alter ecosystems, displace native species, change community structures and food webs, and alter fundamental processes, such as nutrient cycling and sedimentation (Molnar et al. 2008). In the case of fish species, decline of commercial stocks due to direct predation or competition for resources (food or space) is the presumed mechanism of negative impact (Katsanevakis et al. 2014). Therefore, the impact of *C. regalis* in the local ecosystem should be studied in detail as soon as possible.

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